Effect of Heart Work and Insulin on the Incorporation of [^{14}C]Glucose into Hexose Phosphates, Uridine Diphosphate Glucose and Glycogen in the Normal and Insulin-Deficient Perfused Rat Heart under Working and Non-Working Conditions

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1. The specific radioactivities of glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, UDP-glucose and glycogen, derived from [^{14}C]glucose, were determined in the normal and insulin-deficient (streptozotocin-diabetic and anti-insulin-serum-treated) perfused non-working and working rat heart. 2. The specific radioactivities of all glucose metabolites reached a plateau after about 10 min, except that for glycogen, which increased slightly but steadily over the whole observation period of 30 min. 3. The specific radioactivities of fructose 6-phosphate, UDP-glucose and glycogen were significantly lower in the streptozotocin-diabetic heart than in the normal heart. 4. Mechanical work in the normal rat heart increased the specific radioactivities of glucose 1-phosphate, UDP-glucose and glycogen, but had little or no effect on those of glucose 6-phosphate and fructose 6-phosphate. 5. In the normal heart insulin strongly increased the specific radioactivities of all glucose metabolites under all conditions tested. The maximum values achieved in the normal working heart in the presence of insulin were only about 15–20% above those in the normal non-working heart in the presence of insulin for the phosphorylated intermediates and about 40% above for glycogen. 6. In the streptozotocin-diabetic heart, work restored the specific radioactivities of all glucose metabolites to about normal values. 7. In the streptozotocin-diabetic heart insulin strongly increased the specific radioactivities of the direct glycogen precursors glucose 1-phosphate and UDP-glucose; the effect of insulin on glucose 6-phosphate and fructose 6-phosphate was less marked. These results confirm previous findings that the primary metabolic lesion in diabetic heart muscle is a defect of glycogen synthesis. The specific radioactivity of glycogen itself was increased sixfold. 8. Under all conditions tested the specific radioactivity of glucose 1-phosphate was always found to be higher than that of glucose 6-phosphate. This indicated either compartmentation of a small but metabolically very active pool of glucose 6-phosphate, or the existence of a hitherto unknown pathway of metabolism in which glucose 1-phosphate is the primary reaction product. For a number of reasons the authors prefer the first explanation, which could also account for the observation that in the perfused normal working and non-working heart the specific radioactivity of fructose 6-phosphate was always found to be higher than that of glucose 6-phosphate. This difference disappeared or was reversed in the rat hearts rendered insulin-insufficient by either streptozotocin or anti-insulin-serum treatment.

Those interested in the mode of action of insulin in the enhancement of glycogen synthesis in skeletal and heart muscle have always wanted to know the specific radioactivities of the phosphorylated hexose intermediates and UDP-glucose in these tissues in normal and diabetic conditions. However, so far very few reliable data are available, because of the lack of a sufficiently sensitive method for separation and deter-

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mination of these compounds, some of which (glucose 1-phosphate or UDP-glucose) occur in only small amounts. Such a method has now been developed (Thomas & Blanshard, 1974) and been applied to the determination of the above-mentioned sugar phosphates in the perfused normal and streptozotocin-diabetic rat heart, a preparation that has the added advantage of allowing the study of the metabolic conditions in the non-working and working states. Comparison of the specific radioactivities at the

plateau level showed that the specific radioactivity of glucose 1-phosphate was always higher than that of glucose 6-phosphate. The bearing of this finding and the increase in specific radioactivities of glucose 6-phosphate, glucose 1-phosphate, fructose 6-phosphate and UDP-glucose by heart work and insulin are discussed in relation to the hypothesis of two glucose 6-phosphate pools.

Experimental

Materials

Animals. Male albino rats (280–320g) of the Sprague–Dawley strain maintained on a standard laboratory diet (Thompson rat cubes; Thompson, Birmingham, U.K.) were used.

Chemicals. Unless otherwise stated, the chemicals used were of analytical grade, generally purchased from BDH Chemicals, Poole, Dorset, U.K. Ion-exchange resins were obtained from Bio-Rad Laboratories, St. Albans, Herts., U.K. Reference compounds, e.g. glucose 6-phosphate, glucose 1-phosphate, fructose 6-phosphate, UDP-glucose etc., were obtained from Boehringer Corp., London W.5, U.K. Rabbit liver glycogen used as standard was obtained from BDH Chemicals. Streptozotocin [2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose] was given by Upjohn Co., Kalamazoo, MI, U.S.A. Crystalline insulin (glucagon-free) from Burroughs Wellcome and Co., Beckenham, Kent, U.K., was dissolved in 3.3M-HCl to yield a stock solution of 20 i.u./ml, which was stored at -20°C in 1 ml samples. Chromatographically pure $[\text{U-}^{14}\text{C}]$ -glucose 6-phosphate, $[\text{U-}^{14}\text{C}]$ -glucose 1-phosphate, $[\text{U-}^{14}\text{C}]$ -fructose 6-phosphate and UDP- $[\text{U-}^{14}\text{C}]$ -glucose were from The Radiochemical Centre, Amersham, Bucks., U.K.

Methods

Heart perfusion. The perfusion technique and apparatus for isolated rat hearts were essentially those described by Chain *et al.* (1969), based on the method of Neely *et al.* (1967). The perfusion medium, Krebs & Henseleit (1932) bicarbonate buffer, pH 7.4, equilibrated with $\text{O}_2 + \text{CO}_2$ (95:5) and containing glucose (11.1 mM; specific radioactivity approx. 500000 d.p.m./ μmol), was maintained at 37°C and used in all experiments. The details of the conditions and methods of monitoring heart perfusion for stable performance, together with the methods of sample collection, were as described by Chain *et al.* (1969). Working heart preparations were perfused via the left atrium. The aortic output (measured continuously with a glass flowmeter in line; G. A. Planton and Co., Croydon, Surrey, U.K.) was used as an index of the stability of the work performance. In the Langendorff perfusion system 50 ml of perfusion fluid was recircu-

lated with a perfusion pressure of 9.8 kPa (100 cmH₂O). In the working heart preparation 50 ml of perfusion liquid was recirculated at an atrial pressure of 1.96 kPa (20 cmH₂O); the left ventricle ejected 40–60 ml of perfusate/min against a hydrostatic pressure of 9.8 kPa (100 cmH₂O). A drip-through Langendorff pre-perfusion period of 15 min was used in both types of perfusion. For analysis the heart was frozen between aluminium clamping blocks cooled in liquid N₂ (Wollenberger *et al.*, 1960). The frozen muscle was then powdered in a percussion mortar at liquid-N₂ temperature. This powder was used for the determination of glycogen and tissue hexose phosphate intermediates.

Performance of the heart and the effect of insulin was checked by comparing the values of glucose uptake of normal, diabetic and insulin-treated hearts.

The specific radioactivity of the glucose in the perfusate, determined before and after 30 min perfusion, showed no significant difference. The results obtained for the specific radioactivity of the intracellular glucose were very unreliable owing to the contamination by glucose in the perfusate.

Preparation of streptozotocin-diabetic and anti-insulin-serum-treated rats. Chronic streptozotocin-diabetic rats were prepared by injection of 65 mg of streptozotocin/kg body wt. into the tail vein. These animals had free access to water and food, and were used 7 days later. All rats lost weight during this period, and had a blood glucose concentration greater than 350 mg/100 ml, as determined by the glucose oxidase method (Marks & Lloyd, 1963). Anti-insulin serum was prepared as described by Mansford (1967). Serum was injected into the femoral vein 1 h before excision of the heart. Blood glucose concentration at the time of excision was over 350 mg/100 ml.

Specific radioactivities. The specific radioactivities were expressed as d.p.m./ μmol of metabolite. The specific radioactivity of the glucose in the perfusion liquid was kept as near to 500000 d.p.m./ μmol as possible. It was determined for each experiment, and any variations encountered (which never exceeded $\pm 10\%$) were corrected by reference to the standard value in the calculation of the specific radioactivities of the glucose metabolites.

Determination of specific radioactivity of glycogen. Glycogen was extracted from a portion of the frozen perfused-heart powder by the method of Good *et al.* (1933). It was purified by reprecipitation with 66% (v/v) ethanol. After hydrolysis with Diazyme (Cambrian Chemicals, Croydon, Surrey, U.K.) in 0.1 M-sodium acetate buffer, pH 5.0, in a scintillation vial, glucose was determined by the glucose oxidase method (Marks & Lloyd, 1963). The radioactivity of another portion of the hydrolysed glycogen was counted in a Packard model 3003 liquid-scintillation counter with the use of 10 ml of scintillant (Scales, 1967).

Chromatographic separation of hexose phosphates and UDP-glucose and the determination of specific radioactivity. Frozen powdered hearts [0.6–0.8 g in 20 ml of ice-cold 66% (v/v) ethanol] were homogenized in a Vortex mixer. After centrifugation the residue was re-extracted with a further 20 ml of cold 66% ethanol, again homogenized, and then centrifuged. The combined ethanolic supernatants were then evaporated to dryness *in vacuo* at room temperature in a desiccator containing anhydrous CaCl_2 . The residue was dissolved in 1 ml of water and the solution was passed through a column (15 cm $\times$ 0.4 cm internal diam.) of the sulphonic resin Dowex 50 X8 (H^+ form) and eluted with 10 ml of water. The eluate was freeze-dried and taken up in 1 ml of 5M-sodium borate solution for chromatographic analysis.

Chromatographic separation and determination of specific radioactivity of glucose 6-phosphate, glucose 1-phosphate, fructose 6-phosphate and UDP-glucose. The automated method for the separation of sugar phosphates described by Thomas & Blanshard (1974) was used with some improvement to suit the experimental conditions needed for both colour and radioactivity measurements. An anion-exchange polystyrene quaternary ammonium Ag-1 X4 (400 mesh, Cl^- form resin column and a programmed sodium borate/sodium acetate buffer gradient were used.

A continuous-flow orcinol reaction was used as a column-effluent detector for sugars. The coloured reaction products of sugars were monitored at 420 nm with a Bausch and Lomb Spectronic 20 instrument (Applied Research Laboratories, Luton, Beds., U.K.) modified to take a 15 mm flow cell. The column effluent was split and one stream directed to a fraction collector for radioactivity measurement or re-analysis.

Results

Chromatographic separation of standard mixtures of sugar phosphates has been shown (Thomas & Blanshard, 1974). The amount of each compound separated was determined by integrating the area of

Table 2. *Elution times of pure compounds run on the chromatographic system*

A mixture of the pure standard compounds in borate buffer was run in the chromatographic system described in the text, and the times to peak maxima for the elution of various compounds that gave well-defined peaks are shown. The compounds containing no sugar residues (lactate, pyruvate and citrate) were observed by using ^{14}C -labelled pure reference compounds and monitoring their elutions with a flow scintillation spectrometer. The elution times are measured from the breakthrough of unrelated materials in the samples.

Compound	Elution time (min)	Compound	Elution time (min)
Mannose	1	Rib-1-P	49
Sucrose	4	Glc-6-P	55
Trehalose	7	Fru-6-P	61
Ribose	9	Rib-5-P	63
Lactate	15	Cyclic AMP	71
Pyruvate	16	AMP	85
Arabinose	17	Citrate	98
Fructose	20	Glc-1,6- P_2	110
Galactose	25	Fru-1,6- P_2	119
Glucose	30	UDP-glucose	134
Glc-1-P	40	ADP	145
Man-1-P	44	ATP	166
Gal-1-P	45	UTP	182

the peak obtained. The value was then calculated by comparing with the area resulting from a known concentration of pure compound. The orcinol colour response was linear.

The recoveries of standard mixtures added to heart extracts are shown in Table 1, and were in the range 96–103% $\pm$ 7%. This shows that nothing in the heart extract interferes with the yield of colour reaction. In the determinations of specific radioactivities of sugar phosphates obtained with ^{14}C -labelled glucose as substrate it was necessary to establish that both radioactivity and colour peaks were entirely attributable to the compound under study. The peaks of interest were collected and the fractions containing the centre of each peak were re-run through the chromatographic system. Specific radioactivities of sugar phosphates were determined from the re-collected individual peaks. The absence of free glucose was confirmed by using glucose oxidase (Das *et al.*, 1971). The re-collected individual peak fractions were then hydrolysed with 1M-HCl at 100°C for 4 h and again chromatographed. In each case the radioactivity and colour appeared as single peaks with elution times corresponding to the parent sugars, i.e. glucose or fructose. The specific radioactivity of the re-collected sugars was within $\pm$ 5% of the original specific radioactivity of the sugar phosphates that had been re-run. The possibility that the radioactivity

Table 1. *Recoveries of standard compounds added to rat heart extracts*

Standard compounds were added to half of the heart extracts and the sugar phosphates were determined in both the extracts. Compounds originally present in the heart extracts were subtracted from the total amount to measure the recovery of the standard. The results are expressed as means $\pm$ S.D. for five samples.

Compound	Recovery (%)
Glucose	103 $\pm$ 4
Glucose 1-phosphate	98 $\pm$ 7
Glucose 6-phosphate	99 $\pm$ 9
UDP-glucose	96 $\pm$ 8

Fig. 1. Chromatogram of radioactive standards added to unlabelled rat heart extracts

Radioactive standard sugar phosphate mixture was added to heart extract and run through the chromatographic system described in the text. The column effluent was split and a proportion directed to a fraction collector for radioactivity measurement. Radioactivity of the fraction was plotted in the orcinol colour chart to show the coincidence of the radioactivity peaks (-----) with the corresponding colour peaks (—).

at the position of glucose 6-phosphate and glucose 1-phosphate is due to a non-glucose radioactive contaminant was eliminated by following the method of Das *et al.* (1971). In some cases concentrations of glucose 6-phosphate, glucose 1-phosphate, fructose 6-phosphate and UDP-glucose in the re-chromatographed individual sugar peaks were checked by enzymic determinations (Das *et al.*, 1971), and the results correlated well with those obtained from the orcinol colour reactions.

Time to peak maxima for the elution of various compounds that have been run as well-defined peaks on the chromatographic system are shown in Table 2. Metabolic products of glucose containing no sugar residues, e.g. lactate, pyruvate and citrate, were observed by using ¹⁴C-labelled pure reference compounds and monitoring their elution with a flow scintillation spectrometer. The times are measured from the appearance of unretarded material in the samples. Radioactive sugar phosphate standards, when added to the unlabelled heart extract, and the radioactivity of the peaks were measured in fractionally collected sugar phosphates as indicated in Fig. 1 legend. The radioactive sugar phosphate peaks coincided with the orcinol colour peaks, as shown in Fig. 1. Elution volumes are not relevant to a

programmed gradient system, and the appearance times are independent of small changes in the eluent flow rate. The first objective was obviously to establish the time of the perfusion period when the specific radioactivities of the glucose metabolites studied (glucose 6-phosphate, glucose 1-phosphate, fructose 6-phosphate, UDP-glucose and glycogen) had reached equilibrium. To this end the specific radioactivities of these metabolites were determined at different times during the perfusion period in the rat heart in the non-working state (Langendorff perfusion), where the metabolic rates are lower than in the working state. Determinations were made in the normal rat heart in the presence and in the absence of insulin (Figs. 2 and 3) and in the streptozotocin-diabetic rat heart (Fig. 4). In all cases a plateau of the specific radioactivities was reached after about 10 min, except that for glycogen, which increased slightly but steadily throughout the whole perfusion period. Insulin caused a marked increase in the specific radioactivities of all the glucose metabolites studied. In the streptozotocin-diabetic heart all specific radioactivities were lower than in the normal heart. Tables 3 and 4 contain values of the specific radioactivities of the glucose metabolites studied, determined under different conditions after 30 min of perfusion, i.e.

Fig. 2. Variation with time of the specific radioactivities of hexose phosphates, UDP-glucose and glycogen of normal Langendorff perfused heart

All hearts were pre-perfused without recirculation at 9.8 kPa (100 cmH₂O) aortic pressure for 15 min with 11.1 mM-glucose. Then perfusion was switched to recirculation perfusion for 5–30 min by the Langendorff aortic technique with 50 ml recirculation volume. Ethanolic extracts of the heart were purified and the specific radioactivities of glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, UDP-glucose and glycogen were determined as described in the Experimental section. Specific radioactivities of the compounds were calculated by taking perfusate glucose specific radioactivity to be 500 000 d.p.m./μmol. The values are means of the three determinations at each time-interval. ●, Glucose 1-phosphate; ■, glucose 6-phosphate; ○, fructose 6-phosphate; □, UDP-glucose; ▲, glycogen.

after a time-period when, on the basis of the preceding experiments, complete equilibration can be assumed to have been reached.

In the normal heart, work did not significantly increase the specific radioactivities of glycogen and its immediate precursors. Insulin in the non-working heart strongly increased the specific radioactivities of all intermediates and glycogen, the former by a factor of almost 2, the latter by a factor of almost 7. Work led to a further increase of these values by only 15–20% in the case of the phosphorylated intermediates and about 30% in the case of glycogen.

In the streptozotocin-diabetic non-working heart the values for the specific radioactivities of all glucose metabolites and glycogen are considerably lower (50–65%) than those in the normal non-working heart, except for that of glucose 6-phosphate, which is decreased by only about 15%.

Work restored the specific radioactivities of the sugar phosphates and glycogen in the heart of streptozotocin-diabetic rats to normal or a little above normal values. This extends previous observations that mechanical work can reverse some of the metabolic defects, such as glucose uptake and oxidation,

observed to occur in the non-working streptozotocin-diabetic rat heart (Chain *et al.*, 1969).

Addition of insulin to the perfusion fluid of the non-working streptozotocin-diabetic rat heart strongly increased the specific radioactivities of glucose 1-phosphate, glucose 6-phosphate and UDP-glucose to almost normal values. The final values for the specific radioactivities of fructose 6-phosphate and glycogen under these conditions were lower (by about half) than those for normal non-working insulin-treated hearts, but the percentage increase of these values under the influence of insulin was about the same.

Addition of insulin to the perfusion fluid of the working streptozotocin-diabetic heart led to a further small increase in the specific radioactivities of glucose 1-phosphate, glucose 6-phosphate and UDP-glucose, and a sixfold increase in the specific radioactivity of glycogen, bringing it to almost normal. The specific radioactivity of fructose 6-phosphate remained, however, far below that found in the normal working rat heart.

It was decided to compare the specific radioactivities of glucose intermediates and glycogen in the streptozotocin-diabetic rat heart with those

Fig. 3. Variation with time of the specific radioactivities of hexose phosphates, UDP-glucose and glycogen of normal Langendorff perfused heart treated with insulin

Normal rat hearts were perfused for 5–30 min with 11.1 mM-glucose plus 10 m-i.u. of insulin/ml by the Langendorff aortic route at 9.8 kPa (100 cmH₂O) pressure and 50 ml recirculation volume. Ethanolic extracts of freeze-clamped heart tissue were purified and the specific radioactivities of the hexose phosphates, UDP-glucose and glycogen were determined as described in the Experimental section. Specific radioactivities of the compounds were calculated by taking perfusate glucose specific radioactivity to be 500 000 d.p.m./μmol. The values are means of three determinations at each time-interval. ●, Glucose 1-phosphate; ■, glucose 6-phosphate; ○, fructose 6-phosphate; □, UDP-glucose; ▲, glycogen.

Fig. 4. Variation with time of the specific radioactivities of hexose phosphates, UDP-glucose and glycogen of streptozotocin-diabetic Langendorff perfused heart

All perfusions were carried out by recirculation for 5–30 min by the Langendorff aortic technique at 9.8 kPa (100 cmH₂O) pressure. Diabetic rats were used 7 days after the intravenous injection of 65 mg of streptozotocin/kg. Ethanolic extracts of freeze-clamped heart tissue were purified and the specific radioactivities of the hexose phosphates, UDP-glucose and glycogen were determined as described in the Experimental section. Specific radioactivities of the compounds were calculated by taking perfusate glucose specific radioactivity to be 500 000 d.p.m./μmol. Results are the means of three determinations at each time-interval. ●, Glucose 1-phosphate; ■, glucose 6-phosphate; ○, fructose 6-phosphate; □, UDP-glucose; ▲, glycogen.

found for these compounds in non-working and working rat hearts rendered insulin-deficient by prior treatment of the animals with anti-insulin serum. The values obtained are presented in Table 4. In the non-working anti-insulin-serum-treated rat heart the specific radioactivities of all glucose metabolites were similar to those found in the streptozotocin-diabetic heart, i.e. they were all much lower in comparison with the normal control, except that for glucose 6-phosphate, which, as in the streptozotocin-diabetic heart, did not change.

Addition of insulin to the perfusion fluid of the anti-insulin-treated non-working heart restored the specific radioactivities of all compounds to values similar to those in the normal non-working heart, a response that was not observed with glycogen in the streptozotocin-diabetic heart.

The effect of work on overcoming the insulin insufficiency manifests itself particularly strikingly in the anti-insulin-serum-treated hearts. The specific radioactivities of all metabolites investigated were brought back to normal values. Addition of insulin to the perfusion fluid caused a further increase in the specific radioactivities of all the metabolites to values similar to those found in the normal working heart in the presence of insulin, i.e. to considerably higher values than those found in the streptozotocin-diabetic rat heart in the presence of insulin. It is clear that the working rat heart rendered insulin-deficient by treatment with anti-insulin serum reacts better to

Table 3. Specific radioactivities of phosphorylated glucose intermediates and glycogen in the perfused Langendorff and working heart of normal and streptozotocin-diabetic rats in the presence and in the absence of insulin

All hearts were pre-perfused for 15 min at 9.8 kPa (100 cmH₂O) aortic pressure, followed by recirculation perfusion for 30 min under the condition specified [Langendorff heart, aortic pressure 9.8 kPa (100 cmH₂O); working heart, atrial pressure 1.96 kPa (20 cmH₂O)]. Diabetic rats were used 7 days after a single intravenous dose of 65 mg of streptozotocin/kg. The substrate was 11.1 mM-glucose; insulin, when used, was at 10 m-i.u./ml. Ethanolic extracts of the freeze-clamped hearts were purified and the specific radioactivities of glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, UDP-glucose and glycogen were determined as described in the text. Results are given as the ratios of specific radioactivities of the glucose metabolite to that of the perfusate glucose (means ± S.E.M. for the three hearts).

Intermediate	Normal rat heart				Streptozotocin-diabetic heart			
	Langendorff		Working		Langendorff		Working	
	No insulin	Insulin	No insulin	Insulin	No insulin	Insulin	No insulin	Insulin
Glc-1-P	0.409 ±0.016	0.723* ±0.120	0.541 ±0.008	0.891* ±0.042	0.295 ±0.066	0.668* ±0.086	0.548‡ ±0.074	0.771* ±0.112
Glc-6-P	0.209 ±0.024	0.373* ±0.046	0.241 ±0.053	0.492* ±0.062	0.166 ±0.023	0.396* ±0.054	0.318‡ ±0.063	0.493 ±0.079
Fru-6-P	0.316 ±0.039	0.677* ±0.065	0.380 ±0.074	0.702* ±0.051	0.135‡ ±0.012	0.355* ±0.052	0.306‡ ±0.060	0.365 ±0.028
UDP-Glc	0.276 ±0.025	0.523* ±0.082	0.349 ±0.065	0.615* ±0.098	0.090‡ ±0.012	0.455* ±0.060	0.348‡ ±0.065	0.598* ±0.099
Glycogen	0.012 ±0.002	0.083* ±0.006	0.019 ±0.005	0.124* ±0.014	0.006‡ ±0.002	0.036* ±0.005	0.017‡ ±0.005	0.105* ±0.008

* Significant difference ($P \leq 0.05$) between results for no-insulin- and insulin-treated hearts.

† Significant difference ($P \leq 0.05$) between results for normal and streptozotocin-treated Langendorff hearts.

‡ Significant difference ($P \leq 0.05$) between results for working and Langendorff hearts.

Table 4. *Specific radioactivities of glucose intermediates and glycogen in the perfused Langendorff and working heart of anti-insulin-serum-treated rats in the presence and in the absence of insulin*

Hearts were perfused for 30 min in a recirculation system with 50 ml of buffer containing 11.1 mM-glucose, plus 10 m-i.u. of insulin/ml where indicated. Langendorff hearts were at 9.8 kPa (100 cmH₂O) aortic pressure, working hearts at 1.96 kPa (20 cmH₂O) atrial pressure. Ethanol extracts of the freeze-clamped hearts were purified and specific radioactivities of hexose phosphates, UDP-glucose and glycogen were determined as described in the text. Results are given as the ratio of specific radioactivities of the compound and that of the perfusate glucose (means $\pm$ S.E.M. for the three hearts).

Intermediate	Langendorff		Working	
	No insulin	Insulin	No insulin	Insulin
Glc-1-P	0.321 ± 0.052	0.851* ± 0.093	0.603‡ ± 0.081	0.983* ± 0.150
Glc-6-P	0.202 ± 0.021	0.662* ± 0.071	0.482‡ ± 0.038	0.712* ± 0.111
Fru-6-P	0.165† ± 0.032	0.411* ± 0.050	0.428‡ ± 0.041	0.551 ± 0.065
UDP-Glc	0.153† ± 0.021	0.690* ± 0.072	0.525‡ ± 0.067	0.705* ± 0.061
Glycogen	0.010† ± 0.003	0.080* ± 0.009	0.101‡ ± 0.012	0.155* ± 0.020

* Significant difference ($P \leq 0.05$) between results for no-insulin- and insulin-treated hearts.

† Significant difference ($P \leq 0.05$) between results for normal and anti-insulin-serum-treated Langendorff hearts.

‡ Significant difference ($P \leq 0.05$) between results for working and Langendorff hearts.

insulin than does the streptozotocin-diabetic working rat heart.

Discussion

The most noteworthy result of the investigations reported above is that in all cases the specific radioactivity of glucose 1-phosphate was invariably found to be higher than that of glucose 6-phosphate. This phenomenon was previously observed in rat liver after intravenous injection of [U-¹⁴C]glucose (Das *et al.*, 1971) and in pigeon liver homogenates after incubation with [U-¹⁴C]glucose (Das & Sie, 1972). There are two possible explanations. If, according to the conventional concepts of glucose metabolism, the first reaction product of glucose is glucose 6-phosphate, it must be assumed that there exists a small glucose 6-phosphate pool, separated from the rest of the glucose 6-phosphate in a compartment, for instance located on a membrane, which is transformed into glucose 1-phosphate, UDP-glucose and glycogen. Alternatively, a pathway of glucose metabolism different from the conventional one could be envisaged in which glucose 1-phosphate is the first reaction product.

Another strange observation, for which we cannot at present offer any explanation, is that in the normal rat heart in the working and non-working states the specific radioactivity of fructose 6-phosphate was always found to be higher than that of glucose 6-phosphate, whereas in the streptozotocin-diabetic and anti-insulin-serum-treated heart, in the working or

non-working state, there was either no difference between the specific radioactivities of fructose 6-phosphate and glucose 6-phosphate, or the latter had a higher specific radioactivity than the former.

We are also unable to offer an explanation why the combined specific radioactivities of glucose 6-phosphate and fructose 6-phosphate obtained by this method are lower than the values reported by others (Denton & Randle, 1967; Neely *et al.*, 1972). As we are convinced by the effectiveness of our separation technique, it is difficult to explain the differences between the two results.

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